Institute Report No. 297

Mutagenic Potential of BALLPOWDER® in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test

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GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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The mutagenic potential of BALLPOWDER® was assessed by using the Ames Salmonella/ Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate in both the presence and absence of metabolic activation. The test compound was not mutagenic under conditions of this test.

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ABSTRACT

The mutagenic potential of BALLPOWDER® was assessed by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. Tester strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to doses ranging from 5 mg/plate to 0.0016 mg/plate in both the presence and absence of metabolic activation. The test compound was not mutagenic under conditions of this test.

Key Words: Mutagenicity, Genetic Toxicology, Ames Test, BALLPOWDER®

PREFACE

TYPE REPORT: Ames Test GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010

PROJECT/WORK UNIT/APC: #3E162720A835/180/TLB0

GLP STUDY NUMBER: 84041

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MS

PRINCIPAL INVESTIGATOR: Steven K. Sano, BA, SGT

CO-PRINCIPAL INVESTIGATOR: MAJ Earl W. Morgan, DVM, VC

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, tissues, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: BALLPOWDER®

INCLUSIVE STUDY DATES: 26 November - 14 December 1984

OBJECTIVE:

The objective of this study was to determine the mutagenic potential of BALLPOWDER® (50/50 blend of lots BAJ-47670 and BAJ-47671, LAIR Code TA045) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

ACKNOWLEDGMENTS

SP4 John R. Ryabik, BS; SP4 Paul B. Simboli, BS; and Mr. John Dacey provided research assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP study number 84041 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

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MAJ, MSC
Study Director

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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 28 September 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Statement of Compliance

1. This is to certify that the protocol for GLP Study 84Ø41 was reviewed on 18 September 1984.

2. The institute report entitled "Mutagenic Potential of Ballpowder in the Ames Salmonella/Mammalian Microsome Mutagenicity Test," Toxicology Series 1Ø6, was audited on 26 September 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Mutagenic Potential of BALLPOWDER® in the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test--
Sano *et al*

INTRODUCTION

Nitroguanidine is a primary component of US Army triple-base propellants and is now being produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions manufacturing facilities, reviewed the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products. A genetic and acute mammalian toxicity profile of BALLPOWDER®, a fielded nitrocellulose-based propellant (Cartridge 5.56 mm, Ball, M193), was also requested as a baseline against which future formulations will be compared.

The Ames *Salmonella*/Mammalian Microsome Mutagenicity Test is a short-term screening assay that utilizes histidine auxotrophic mutant strains of *Salmonella typhimurium* to detect those compounds which are potentially mutagenic in mammals. A mammalian microsomal enzyme system is incorporated in the assay to increase sensitivity by simulating *in vivo* metabolic activation of the test compound. The Ames Test is an inexpensive yet highly predictive and reliable assay for detecting mutagenic activity and thus carcinogenic potential (2).

Objective of the Study

The objective of this study was to determine the mutagenic potential of BALLPOWDER® (50/50 blend of lots BAJ-47670 and BAJ-47671, LAIR Code TA045) by using the Ames *Salmonella*/Mammalian Microsome Mutagenicity Test.

MATERIALS AND METHODS

Test Compound

Product name: WC 844 Double-base Spheroidal Propellant

LAIR Code number: TA045

Physical state: Solid

Source: Badger Army Ammunition Plant
Baraboo, WI 53913

Storage: BALLPOWDER® (50/50 blend of lots BAJ-47670 and BAJ-47671) was received from Badger Army Ammunition Plant (AAP) on 6 September 1984 and assigned the LAIR Code number TA045. The test compound was stored at room temperature (21°C) until used.

Chemical Properties/Analysis: Data provided by Badger AAP characterizing the chemical composition and purity of the test material are presented in Appendix A.

Test Solvent

The test compound and the positive control chemicals were dissolved in grade I dimethyl sulfoxide (lot 113F-0450) obtained from Sigma Chemical Co. (St. Louis, MO).

Chemical Preparation

BALLPOWDER® was stored at room temperature (21°C) until used. On the day before dosing, 300 mg of the test compound was measured into a sterile vial and again stored at room temperature. On the day of dosing, the 300 mg of the test compound that had been measured into a sterile vial was dissolved in 6 ml grade I dimethyl sulfoxide to achieve a 5% (w/v) solution. Aliquots of this solution were used to dose the test plates.

Test Strains

Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538, obtained directly from Dr. Bruce Ames, University of California, Berkeley, CA, were used. These strains were maintained in our laboratory at -80°C. Quality controls were run concurrently with the test substance to establish the validity of their special features and to determine the spontaneous reversion rate. Descriptions of the strains,

their geretic markers, and the methods for strain validation are given in the LAIR SOP, OP-STX-1 (3).

Test Format

BALLPOWDER® was evaluated for mutagenic potential according to the methods of Ames *et al* (4). A detailed description of the methodology is given in LAIR SOP, OP-STX-1 (3).

Toxicity Tests

Toxicity tests were conducted to determine a sublethal concentration of the test substance. This toxicity level was found by using minimal glucose agar (MGA) plates, concentrations of BALLPOWDER® ranging from 1.6×10^{-3} mg/plate to 5 mg/plate and approximately 10^8 cells of TA100 per plate. Top agar containing trace amounts of histidine and biotin was placed on the plates. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth on the plates was observed. Since none of the plates showed decreased macrocolony formation (below the level of the spontaneous reversion plates) or an observable reduction in the density of the background lawn, a maximum "limit" dose of 5 mg per plate was used in the mutagenicity assay.

Mutagenicity Tests

The test substance was evaluated over a 1000-fold range of concentrations, decreasing from the minimum toxic level (the maximum or limit dose) by a dilution factor of 5, both with and without 0.5 ml of the S-9 microsome fraction. The S-9 was purchased from Litton Bionetics (Kensington, MD). The optimal titer of this S-9, as determined by Litton Bionetics, was 0.75 mg protein/plate. After all the ingredients were added, the top agar was mixed, then overlaid on MGA plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (5). The water used in this medium and in all reagents came from a Polymetric model 200-3 Water Purifier (Sunnyvale, CA). Plates were incubated upside down in the dark, at 37°C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The average number of revertants at each dose level was compared to the average number of spontaneous revertants (negative control). The spontaneous reversion rate (with and without S-9) was monitored by averaging the counts from two determinations run simultaneously with the test compound assay. The spontaneous reversion rate was determined by inoculating one set of plates before and one set after the test compound assay plates so that any change in spontaneous

reversion rate during the dosing procedure would be detected. This spontaneous reversion rate was also compared with historical values for this laboratory and those cited in Ames *et al* (4). Concurrent sterility and strain verification controls were run. All reagents, test compounds, and media were checked for sterility by plating samples of each on MGA media and incubating them at 37°C with the test plates. The *Salmonella* strains were verified by a standard battery of tests. The following tests were run to determine if:

- Lipopolysaccharide layer (LP) alteration causes growth inhibition in the presence of crystal violet.

- An ampicillin-resistant R factor has allowed growth in strains TA98 and TA100 in the presence of ampicillin-impregnated disks.

- Absence of excision repair mechanism has inhibited growth in the presence of ultraviolet light.

Four known mutagens were tested as positive controls to confirm the responsiveness of the strains to the mutation process. These compounds (benzo[a]pyrene, 2-aminofluorene, 2-aminoanthracene, and N-methyl-N'-nitro-n-nitrosoguanidine) were obtained from Sigma Chemical Co. (St. Louis, MO). The test compound and mutagens were handled during this study in accordance with the standards published in NIH Guidelines for the Laboratory Use of Chemical Carcinogens (DHHS Publication No. (NIH) 81-2385, May 1981).

Data Interpretation

According to Brusick (6), a compound is considered mutagenic if the following criteria are met:

1. For strains TA98 and TA100, a positive dose response (correlated dose response) over three dose concentrations is achieved with at least the highest dose yielding a revertant colony count greater than or equal to twice the spontaneous colony count for the strain. A strong correlated dose response in strain TA100 without a doubling of the individual colony count may also be considered positive.

2. For strains TA1535, TA1537, and TA1538, a correlated dose response over three concentrations is achieved with at least one dose yielding a revertant colony count three times the spontaneous colony count for the strain.

Storage of the Raw Data and Final Report

A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR archives.

Deviations from the Protocol/SOP

None.

RESULTS

On 5 December 1984, the toxicity level determination was performed on BALLPOWDER® (Table 1). For this experiment all sterility, strain verification, and negative controls were normal (Table 2). No toxicity was observed after exposure of the tester strain (TA100) to the highest dose used (5 mg/plate).

Normal results were obtained for all sterility, strain verification, and negative controls during the Ames Test performed on 12-14 December 1984 (Table 3). BALLPOWDER® did not induce any appreciable increase in the revertant colony counts relative to those of the negative control cultures (Table 3).

Individual plate scores for the toxicity test, verification controls, and the mutagenicity test are presented in Appendix B.

TABLE 1: TOXICITY DETERMINATION FOR BALLPOWDER®

GLP STUDY: 84041 7 DEC 84 PERFORMED BY: SANO, MORGAN

TA100 REVERTANT PLATE COUNT

TEST COMPOUND CONCENTRATION	MEAN ±1SD	BACKGROUND LAWN*
NEGATIVE CONTROL	65 ± 9.0	NL
5.0 mg/plate	79 ± 9.2	NL
1.0 mg/plate	97 ±11.5	NL
0.2 mg/plate	92 ± 6.0	NL
0.04 mg/plate	88 ± 2.1	NL
0.008 mg/plate	82 ± 8.1	NL
0.0016 mg/plate	85 ± 4.2	NL

STRAIN VERIFICATION FOR TOXICITY DETERMINATION

	TA100*
HISTIDINE REQUIREMENT	G
AMPICILLIN RESISTANCE	G
UV	NG
CRYSTAL VIOLET SENSITIVITY	NG (12mm)
STERILITY CONTROL	NG

STERILITY CONTROL FOR TOXICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG

* NL=Normal Lawn, G=Growth, NG=No Growth, ST=Slight Toxicity

TABLE 2: STRAIN VERIFICATION AND STERILITY TESTING FOR THE MUTAGENICITY DETERMINATION OF BALLPOWDER®

GLP STUDY: 84041 13 DEC 84 PERFORMED BY: SANO, MORGAN

STRAIN VERIFICATION

	OBSERVATIONS*				
STRAIN	HISTIDINE REQUIREMENT	AMPICILLIN RESISTANCE	UV REPAIR	CRYSTAL VIOLET	CONTROL
TA98	NG	G	NG	NG	NG
TA100	NG	G	NG	NG	NG
TA1535	NG	NG	NG	NG	NG
TA1537	NG	NG	NG	NG	NG
TA1538	NG	NG	NG	NG	NG

STERILITY CONTROL FOR MUTAGENICITY DETERMINATION

MATERIAL TESTED	OBSERVATION*
MINIMAL GLUCOSE AGAR PLATES	NG
TOP AGAR	NG
DILUENT WATER	NG
NUTRIENT BROTH	NG
TEST COMPOUND (HIGHEST DOSE)	NG
S-9	NG

*G = Growth, NG = No Growth

TABLE 3: MUTAGENICITY ASSAY FOR BALLPOWDER®

REVERTANTS/PLATE (MEAN ± 1 SD)

COMPOUND	DOSE/PLATE	TA98	TA100
		WITHOUT S-9	
NEG CONTROL	0.0µg	16 ± 7.6	110 ± 9.1
MNNG*	2.0 µg	-	2189 ± 210.1
MNNG*	20.0 µg	-	-
TA045	5000 µg	14 ± 2.3	74 ± 5
TA045	1000 µg	15 ± 3.2	61 ± 4.2
TA045	200 µg	14 ± 0.6	66 ± 6.7
TA045	40.0 µg	24 ± 2.3	69 ± 4.2
TA045	8.0 µg	16 ± 0	75 ± 4
TA045	1.6 µg	14 ± 1.5	80 ± 3.6
		WITH S-9	
NEG CONTROL	0.0 µg	18 ± 7.3	86 ± 13
2-AA*	2.0 µg	1049 ± 432.9	1783 ± 208.4
2-AF*	2.0 µg	581 ± 79.9	467 ± 31.8
BP*	2.0 µg	428 ± 50.1	281 ± 104.3
TA045	5000 µg	14 ± 4	77 ± 8.6
TA045	1000 µg	13 ± 1	76 ± 2
TA045	200 µg	18 ± 4	58 ± 4
TA045	40.0 µg	19 ± 3.5	76 ± 6.1
TA045	8.0 µg	20 ± 0.6	59 ± 5
TA045	1.6 µg	11 ± 6.4	74 ± 11.8

* 2-AA = 2-aminoanthracene, 2-AF = 2-aminofluorene, BP = benzo[a]pyrene, MNNG = N-methyl-N'-nitro-n-nitrosoguanidine

TABLE 3 (cont.): MUTAGENICITY ASSAY FOR BALLPOWDER®

REVERTANTS/PLATE (MEAN ± 1 SD)

COMPOUND	DOSE/PLATE	TA1535	TA1537	TA1538
		WITHOUT S-9		
NEG CONTROL	0.0 µg	21 ± 2.1	6 ± 1.4	21 ± 3
MNNG*	2.0 µg	-	-	-
MNNG*	20.0 µg	2070 ± 331.3	-	-
TA045	5000 µg	14 ± 4	5 ± 3.5	17 ± 2.9
TA045	1000 µg	17 ± 5.9	4 ± 0.6	16 ± 3.8
TA045	200 µg	16 ± 3.6	4 ± 1.5	18 ± 7.5
TA045	40.0 µg	14 ± 4.4	4 ± 0.6	21 ± 3.5
TA045	8.0 µg	19 ± 2.5	4 ± 1.5	10 ± 4.4
TA045	1.6 µg	16 ± 1.2	8 ± 4	5 ± 4.5
		WITH S-9		
NEG CONTROL	0.0 µg	12 ± 3.6	6 ± 3.9	18 ± 7.1
2-AA*	2.0 µg	-	210 ± 16.8	1177 ± 85.9
2-AF*	2.0 µg	-	-	562 ± 134.7
BP*	2.0 µg	-	53 ± 13.5	92 ± 10.1
TA045	5000 µg	8 ± 2.5	2 ± 1	15 ± 3.2
TA045	1000 µg	12 ± 1.2	4 ± 2.5	11 ± 5.5
TA045	200 µg	6 ± 2	7 ± 1	12 ± 2
TA045	40.0 µg	10 ± 1.5	7 ± 3.6	11 ± 8.4
TA045	8.0 µg	14 ± 2.1	3 ± 1	20 ± 5.7
TA045	1.6 µg	9 ± 1.2	4 ± 1	9 ± 2.1

* 2-AA = 2-aminoanthracene, 2-AF = 2-aminofluorene, BP = benzo[a]pyrene, MNNG = N-methyl-N'-nitro-n-nitrosoguanidine

DISCUSSION

Certain test criteria must be satisfied before an Ames Test can be considered a valid assessment of a compound's mutagenic potential. First, the special features of the Ames strains must be verified. These features include demonstration of ampicillin resistance, LP (lipopolysaccharide) layer alterations, and DNA excision repair deficiencies. Second, the *Salmonella* strains must be susceptible to mutation by known mutagens. Third, the optimal concentration of the test compound must be determined by treating TA100 with a broad range of doses and observing the potential toxic effects on macrocolonies and microcolonies. If these tests are performed and expected data are obtained, then the results of the Ames Test can be considered valid.

After validation of bacterial strains and selection of optimal sublethal doses, BALLPOWDER® was evaluated in the Ames Test. Criteria for a positive response include both a correlated dose-response relationship over three dose concentrations and a revertant colony count at least two times (TA98 or TA100) or three times (TA1535, TA1537, or TA1538) the spontaneous revertant colony count (6). BALLPOWDER® did not induce the requisite dose-response relationship or the increase in revertant colony counts necessary for a positive response. Thus, the results of this assay indicate that BALLPOWDER® is not mutagenic when evaluated in the Ames Test.

CONCLUSION

BALLPOWDER® was evaluated in the Ames Test, in both the presence and absence of metabolic activation, and did not produce a mutagenic response at the dose levels tested.

REFERENCES

1. Kenyon KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, Maryland: US Army Medical Bioengineering Research and Development Laboratory, 1982. DTIC No. ADA 125591.

2. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the *Salmonella*/Mammalian microsome test: Test of 300 chemicals. Proc Natl Acad Sci USA 1975;72:5135-5139.

3. Ames *Salmonella*/Mammalian Microsome Mutagenicity Test. LAIR Standard Operating Procedure OP-STX-1, Letterman Army Institute of Research, Presidio of San Francisco, California, 15 November 1983.

4. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/Mammalian microsome mutagenicity test. Mutat Res 1975;31:347-364.

5. Vogel HJ, Bonner DM. Acetylornithinase of *E. coli*: Partial purification and some properties. J Biol Chem 1956;218:97-106.

6. Brusick D. Genetic Toxicology. In: Hayes AW, ed. Principles and Methods of Toxicology. *New York:* Raven Press, 1982: 223-272.

Appendix A: CHEMICAL DATA SHEET

PROPELLANT DESCRIPTION SHEET

REPORTS CONTROL SYMBOL: EXEMPT - PARA 7-2e AR 335-15

TO	FROM	DATE
	Badger Army Ammunition Plant Baraboo, Wisconsin 53913	10 August 1984

DA LOT NUMBER: 50/50 blend of lots BAJ-47670 and BAJ-47671	COMPOSITION NUMBER: WC 844 for Cartridge 5.56 mm, BALL, M193
MFG AT: Badger Army Ammunition Plant	PACKED AMOUNT: LB
CONTRACT NUMBER: DAAA09-73-C-0004	SPECIFICATION NUMBER: MIL-P-3984E w/Amendment 4 and Drawing No. C10542743 Rev. C

NITROCELLULOSE

ACCEPTED BLEND NUMBERS	NITROGEN CONTENT	KI STARCH(65.5°C)	STABILITY (134.5°C)
Nitrocellulose (NC) extracted from excessed Single Base Propellant.	MAX %	MIN	MIN
	MIN %	MIN	MIN
	AVG %	MIN	MIN
NC complied with MIL-N-244A			EXPLOSION HR

MANUFACTURE OF PROPELLANT

_____POUNDS SOLVENT PER POUND NC/DRY WEIGHT INGREDIENTS CONSISTING OF _____ POUNDS ALCOHOL AND _____ POUNDS _____ PER 100 POUNDS SOLVENT. PERCENTAGE REMIX TO WHOLE_____.

TEMPERATURE FROM	TEMPERATURE TO	PROCESS-SOLVENT RECOVERY AND DRYING	TIME DAYS	TIME HOURS

PROPELLANT COMPOSITION / TESTS OF FINISHED PROPELLANT / STABILITY AND PHYSICAL TESTS

CONSTITUENT	% FORMULA	% TOLERANCE	% MEASURED		FORMULA	ACTUAL
Nitroglycerin			10.235	HEAT TEST 120°	Min 60 min	65 min.*
Dinitrotoluene			0.685	No Explosion (HRS)	Min 5	5+*
Diphenylamine			1.105	FORM OF PROPELLANT		
Dibutylphthalate			5.255	Dust&Foreign Mat.		0.02
Nitrocellulose			83.23	Graphite		0.075
Total Volatiles			1.045	Gray. Density		1.008
Moisture and Volatiles			0.895	Nitrogen		13.075
Residual Solvent			0.49			
Calcium Carbonate			0.09			
Sodium Sulfate			0.12			

CLOSED BOMB	LOT NUMBER	TEMP °F	relative impetus	relative force
TEST				
STANDARD			100.00%	100.00%

PROPELLANT DIMENSIONS (INCHES)	SPEC	DIE	FINISHED	mean variation in % of mean dimension SPEC	mean variation in % of mean dimension ACTUAL
LENGTH (L)					
DIAMETER (D)					
PERF DIA (d)					
				PACKED	
				SAMPLED	
WEB DIFFERENCE /STD DEV IN % OF WEB AVERAGE				TEST FINISHED	
				OFFERED	
L:D				DESCRIPTION SHEETS FORWARDED	
D:d					

REMARKS

TYPE OF PACKING CONTAINER

REMARKS

*Tested 29 February 1984.

SIGNATURE OF CONTRACTOR'S REPRESENTATIVE	SIGNATURE OF GOVERNMENT QUALITY ASSURANCE REPRESENTATIVE

Appendix B: INDIVIDUAL PLATE SCORES

BALLPOWDER® (TA045)
TOXICITY TEST
TA100

DOSE/PLATE	5.0 mg	1.0 mg	0.2 mg	0.04 mg
PLATE 1	81	84	91	87
PLATE 2	69	106	98	86
PLATE 3	87	101	86	90
BACKROUND LAWN	NL	NL	NL	NL

DOSE/PLATE	0.008 mg	0.0016 mg	NEG CONTROL
PLATE 1	83	86	74
PLATE 2	89	80	56
PLATE 3	73	88	64
BACKROUND LAWN	NL	NL	

Appendix B (cont.): INDIVIDUAL PLATE SCORES

NEGATIVE CONTROLS FOR MUTAGENICITY TESTS
BALLPOWDER® (TA045)

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
		WITHOUT S-9				
NEG CONTROL (START RUN)	0.0 mg	18	112	19	6	25
		19	111	24	5	19
		8	126	22	5	19
NEG CONTROL (END RUN)	0.0 mg	17	108	19	8	24
		8	101	19	4	23
		28	102	20	5	18
		WITH S-9				
NEG CONTROL (START RUN)	0.0 mg	27	78	11	5	28
		11	97	12	2	16
		27	99	19	10	10
NEG CONTROL (END RUN)	0.0 mg	18	88	9	3	21
		14	87	10	6	11
		12	64	13	12	23

Appendix B (cont.): INDIVIDUAL PLATE SCORES

POSITIVE CONTROLS FOR MUTAGENICITY TESTS
BALLPOWDER® (TA045)

COMPOUND*	DOSES/PLATE	TA98	TA100	TA1535	TA1537	TA1538
2-AA	2.0 µg	1239	1605	-	229	1162
		1355	2012	-	199	1099
		554	1731	-	201	1269
2-AF	2.0 µg	590	457	-	-	418
		497	503	-	-	685
		656	442	-	-	583
BP	2.0 µg	486	303	-	66	98
		396	167	-	53	97
		403	372	-	39	80
MNNG	2.0 µg	-	2395	-	-	-
		-	2197	-	-	-
		-	1975	-	-	-
MNNG	20.0 µg	-	-	2183	-	-
		-	-	1697	-	-
		-	-	2330	-	-

* 2-AA = 2-aminoanthrancene, 2-AF = 2-aminofluorene, BP = benzo[a]pyrene, MNNG = N-methyl-N'-nitro-n-nitrosoguanidine

Appendix B (cont.): INDIVIDUAL PLATE SCORES

MUTAGENICITY TESTS
BALLPOWDER® (TA045)

WITHOUT S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TA045	5.0 mg	17	74	15	1	15
		13	69	18	5	15
		13	79	10	8	20
TA045	1.0 mg	11	62	10	4	14
		16	56	21	5	20
		17	64	19	4	13
TA045	0.2 mg	14	69	13	4	10
		13	70	15	2	19
		14	58	20	5	25
TA045	0.04 mg	23	66	19	4	21
		27	74	11	4	18
		23	68	12	5	25
TA045	0.008 mg	16	75	19	4	13
		16	79	21	6	12
		16	71	16	3	5
TA045	0.0016 mg	14	84	15	4	10
		16	77	15	7	5
		13	79	17	12	1

Appendix B (cont.): INDIVIDUAL PLATE SCORES

MUTAGENICITY TESTS
BALLPOWDER® (TA045)

WITH S-9

COMPOUND	DOSE/PLATE	TA98	TA100	TA1535	TA1537	TA1538
TA045	5.0 mg	10	86	8	2	11
		18	75	10	3	17
		15	69	5	1	16
TA045	1.0 mg	13	74	11	4	16
		12	76	13	6	11
		14	78	13	1	5
TA045	0.2 mg	18	57	8	8	12
		14	62	4	7	10
		22	54	6	6	14
TA045	0.04 mg	23	71	10	10	15
		17	75	11	8	16
		17	83	8	3	1
TA045	0.008 mg	20	64	12	2	15
		19	54	16	3	26
		20	59	13	4	18
TA045	0.0016 mg	14	87	10	3	10
		4	64	8	5	11
		16	71	8	4	7

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